

## CLAIMS

1. A method for isolating a polynucleotide that encodes a polypeptide of interest which comprises a signal sequence for secretion or partial secretion, the method comprising the sequential steps of:
- 5 a) providing a DNA or cDNA library from an organism producing the polypeptide of interest, wherein the library is comprised in a circular vector and is produced *in vitro* without ultraviolet irradiation of the component polynucleotides;
  - b) amplifying the library by rolling circle amplification, thereby forming concatamers;
  - c) inserting into the library a DNA fragment comprising a promoterless and secretion  
10 signal-less polynucleotide encoding a secretion reporter;
  - d) introducing the amplified library comprising the inserted DNA fragment into a host cell;
  - e) screening for and selecting a host cell that secretes or partially secretes the active secretion reporter; and
  - f) identifying from the selected host cell the polynucleotide into which the secretion  
15 reporter was inserted, and isolating the polynucleotide;
- wherein steps b) and c) may be performed in any order.
2. The method of claim 1, wherein the DNA or the cDNA library is normalized.
- 20 3. The method of claim 1 or 2, wherein the DNA or the cDNA library is derived from a microorganism.
4. The method of claim 3, wherein the microorganism is a fungus, a filamentous fungus or a yeast.
- 25 5. The method of claim 3, wherein the microorganism is a bacterium.
6. The method of claim 3, wherein the microorganism is an archaeon.
- 30 7. The method of claim 1 or 2, wherein the DNA or the cDNA library is derived from a multicellular organism, preferably from a mammalian cell, most preferably from a human cell.
8. The method of any of claims 1 – 7, wherein the vector comprises at least one restriction enzyme cleavage site and/or at least one *cos* site and/or at least one recombination  
35 recognition site.

9. The method of any of claims 1 - 8, wherein step c) is performed *in vitro*.
10. The method of any of claims 1 - 9, wherein the DNA fragment of claim 1 comprises a  
5 transposon, preferably a MuA transposon.
11. The method of any of claims 1 - 10, wherein the DNA fragment comprises an origin of replication which is functional in the host cell, preferably the origin of replication is functional in *Escherichia coli*, more preferably the origin of replication is a derivative of colE1, oriV,  
10 P15A, or colDF13, and most preferably the origin of replication is colE1.
12. The method of any of claims 1 - 11, wherein the secretion reporter is a protein which, when secreted from the host cell, allows said cell to grow in the presence of a substance which otherwise inhibits growth of said cell.
- 15 13. The method of claim 12, wherein the secretion reporter is a  $\beta$ -lactamase or an invertase.
14. The method of any of claims 1 - 13, wherein the polynucleotide of the DNA-fragment of  
20 a specific target site for proteolytic cleavage.
15. The method of any of claims 1 - 14, wherein the amplified library concatamers are converted to monomers before performing step d).
- 25 16. The method of claim 15, wherein the vector comprises at least one restriction enzyme recognition site, and the concatamers are converted to monomers by restriction enzyme digestion and then circularized by ligation.
17. The method of claim 15, wherein the vector comprises at least one recombination  
30 recognition site, and the concatamers are converted to monomers by circularization through homologous recombination, mediated by the recognition sites and a specific recombinase.
18. The method of any of claims 15 - 18, wherein the monomers are circularized and then treated with a DNA topoisomerase.

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19. The method of any of claims 1 – 14, wherein the vector comprises at least one *cos* site, and wherein subsequent to steps b) and c) the amplified library concatamers are converted to monomers prior to step d) by *cos* site cleavage during phage-packaging.
- 5 20. The method of any of claims 1 – 14, wherein the library is introduced into the host cell as concatamers.
21. The method of any of claims 1 – 20, wherein the host cell is bacterial.
- 10 22. The method of claim 21, wherein the bacterial host cell is an *Escherichia*, *Lactococcus*, *Streptomyces*, *Enterococcus* or *Bacillus* cell, preferably of the species *Escherichia coli*, *Lactococcus lactis*, *Streptomyces griseus*, *Streptomyces coelicor*, *Enterococcus faecalis*, *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus brevis*, *Bacillus circulans*, *Bacillus clausii*, *Bacillus coagulans*, *Bacillus lautus*, *Bacillus lentus*, *Bacillus licheniformis*, *Bacillus*  
 15 *megaterium*, *Bacillus stearothermophilus*, *Bacillus subtilis*, or *Bacillus thuringiensis*.
23. The method of any of claims 1 – 20, wherein the host cell is fungal.
24. The method of claim 23, wherein the fungal host cell is of the genus *Candida*,  
 20 *Kluyveromyces*, *Pichia*, *Saccharomyces*, *Schizosaccharomyces*, *Yarrowia*, *Acremonium*, *Aspergillus*, *Aureobasidium*, *Cryptococcus*, *Filibasidium*, *Fusarium*, *Humicola*, *Magnaporthe*, *Mucor*, *Myceliophthora*, *Neocallimastix*, *Neurospora*, *Paecilomyces*, *Penicillium*, *Piromyces*, *Schizophyllum*, *Talaromyces*, *Thermoascus*, *Thielavia*, *Tolypocladium*, or *Trichoderma*.
- 25 25. The method of claim 23, wherein the fungal host cell is of the species *Saccharomyces cerevisiae*, *Aspergillus aculeatus*, *Aspergillus awamori*, *Aspergillus nidulans*, *Aspergillus niger*, or *Aspergillus oryzae*.
26. The method of any of claims 1 – 20, wherein the host cell is mammalian.
- 30 27. The method of any of claims 1 – 26, wherein the identifying of the polynucleotide in step f) is done by DNA sequencing using at least one primer directed to the DNA fragment of step c), or using at least one primer directed to the vector of step a).
- 35 28. The method of claim 27, where isolating the polynucleotide in step f) is done by utilizing the DNA sequence information obtained.

29. The method of any of claims 1 – 28, wherein the polynucleotide in step f) is isolated from the genome of the organism producing the polypeptide of interest, or from a DNA or cDNA library of the organism.

5 30. The method of any of claims 1 – 29, wherein the polypeptide of interest is an enzyme that is secreted from the host cell.

31. The method of any of claims 1 – 29, wherein the polypeptide of interest is a membrane-bound receptor, preferably a two-component signal (TCS) transduction receptor, and more  
10 preferably a cytokine receptor.

32. The method of any of claims 1 – 29, wherein the polypeptide of interest is a secreted cytokine.

15 33. The method of any of claims 1 – 29, wherein the polypeptide of interest is a polypeptide which elicits an immunogenic response in humans.

34. The method of any of claims 1 – 29, wherein the polypeptide of interest has antimicrobial  
20 activity.

35. The method of any of claims 1 – 29, wherein the polypeptide of interest is a plant pathogenic polypeptide.

25 36. The method of any of claims 1 – 35, wherein an additional step of constructing an expression system is performed, said expression system comprising the polynucleotide isolated in step f).

37. A polynucleotide encoding a polypeptide of interest, wherein said polynucleotide is  
30 isolated by the method of the present invention.

38. A polypeptide of interest which is encoded by a polynucleotide as defined in claim 37.

39. An expression system comprising a polynucleotide as defined in claim 37.

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40. A host cell comprising at least one copy of a polynucleotide as defined in claim 37, or an expression system as defined in claim 39.

41. The host cell of claim 40, wherein at least two copies of the polynucleotide as defined in claim 37 are chromosomally integrated.

42. A process for producing a polypeptide of interest, comprising cultivating a host cell as defined in claim 40 or 41 under conditions suitable for expressing the polynucleotide as defined in claim 37, wherein said host cell secretes the polypeptide encoded by said polynucleotide into the growth medium.

43. The process of claim 42, wherein the polypeptide is an enzyme or a polypeptide having anti-microbial activity.

44. The process of claim 42 or 43, where an additional step of purifying the polypeptide is performed.